Science of the Total Environment 598 (2017) 889–899

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Nature and origin of the violet stains on the walls of a Roman tomb

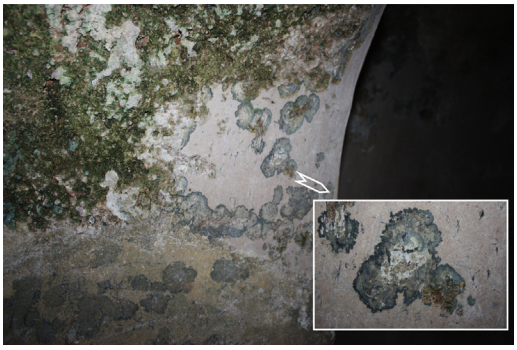
Irene Dominguez-Moñino [a,1], Marta Diaz-Herraiz [a,1], Valme Jurado [a], Leonila Laiz [a], Ana Z. Miller [a], Juan Luis Santos [b], Esteban Alonso [b], Cesareo Saiz-Jimenez [a,*]

[a] Instituto de Recursos Naturales y Agrobiologia, IRNAS-CSIC, Avenida Reina Mercedes 10, 41012 Sevilla, Spain
[b] Escuela Politecnica Superior, Universidad de Sevilla, Virgen de Africa 7, 41011 Sevilla, Spain

HIGHLIGHTS

- The walls and ceiling of the Circular Mausoleum tomb, Spain, are biodeteriorated by microorganisms.
- Violet stains are produced by a strain of *Streptomyces parvus* isolated from the walls.
- The violet pigment is composed of granaticins, compounds soluble in alkaline media.
- The pigment diffuses to the mortar and surrounding substrata during wetting periods.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 February 2017
Received in revised form 3 April 2017
Accepted 4 April 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
Streptomyces
Violet stains
Cyanobacteria
Granaticins
Roman tomb
Biodeterioration

ABSTRACT

The Circular Mausoleum tomb (Roman Necropolis of Carmona, Spain) dates back from the first century AD and is characterized by a dense microbial (phototrophic) colonization on the walls and ceiling. However, some walls exhibited an important number of violet stains of unknown origin. The microbial communities of these violet stains are mainly composed of cyanobacteria, streptomycetes and fungi. A strain of *Streptomyces parvus*, isolated from the walls, produces a violet pigment in culture media. High performance liquid chromatography-mass spectrometry of the culture extracts obtained from this *Streptomyces* revealed the presence of a few granaticins, pigments with a benzoisochromanequinone structure. When metabolically active in the tomb, *S. parvus* synthesizes the pigments that diffuse into the mortar. During rain and/or wetting periods, the pigments are solubilized by alkaline waters and elute from the starting position to the surrounding mortar, enlarging the pigmented area and thus contributing to this exceptional biodeterioration phenomenon.

1. Introduction

Many Roman necropolises are decorated with magnificent mural paintings. The conservation of these paintings is difficult, mainly derived from environmental factors and the opening of the tombs to public visits. One of the most complex phenomena observed in these sites is biodeterioration.

Subsurface environments (caves, necropolises, catacombs, tombs, crypts, etc.) are characterized by their microclimatic conditions, mainly high relative humidity the year round, lower temperatures than outside and poor ventilation (Saiz-Jimenez et al., 2011; Diaz-Herraiz et al., 2014; Fernandez-Cortes et al., 2015; Cañaveras et al., 2015). These conditions

* Corresponding author.
E-mail address: saiz@irnase.csic.es (C. Saiz-Jimenez).
[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.scitotenv.2017.04.017
0048-9697/

promote the growth of Actinobacteria, a common phylum in tombs, with *Nocardia*, *Pseudonocardia* and *Streptomyces* as the most abundant genera (Diaz-Herraiz et al., 2013, 2014). Actinobacteria produce colored biofilms on mural paintings (Agarossi, 1994; Ettenauer et al., 2014) and many species of this phylum are characterized by the production of pigments (Waksman and Lechevalier, 1953; Bérdy, 2005).

The Circular Mausoleum is a small tomb located in the Roman Necropolis of Carmona, Spain. This tomb is actively colonized by photosynthetic microorganisms as reported by Cañaveras et al. (2015). However, these authors did no investigate the nature and origin of the abundant violet stains that can be observed on the walls (Fig. 1). The colored stains have been noticeable since the last quarter of the 20th century and increased over time. The presence of violet stains on mural paintings and walls is rare. They were only reported in two Etruscan tombs whose pigments were not identified (Agarossi, 1994).

A few sampling campaigns were conducted in the Circular Mausoleum to analyze the microbial communities associated with the stains and to know the chemical structure of the violet pigment. To this aim Bacteria, Archaea and Eukaryota were investigated. In different samplings periods we isolated three strains of *Streptomyces* involved in the production of a diffusible violet pigment in laboratory cultures. The isolation of strains that produce violet pigments, either in nature or in the laboratory, together with the identification of the metabolites permitted to disclose the nature of this biodeterioration phenomenon.

2. Methodology

2.1. Sampling of violet stains and cultivation assays

In December 2012, a sampling campaign was conducted in the Circular Mausoleum tomb from the Roman Necropolis of Carmona, Spain. A few violet stains were collected from the walls (Fig. 1) using a sterilized scalpel, and placed into sterile tubes and used for different analyses. Three types of samples were selected: GB1 from green biofilm (near to violet stains), GV2 from an area where green biofilms and violet stains were in close contact, and VS3 from violet stains far from green biofilms. GV2 and VS3 samples were used for molecular analyses and VS3 for bacterial isolation. VS3 sample was processed the day of sampling, suspended in a saline solution and inoculated on Petri plates with a TSA (tryptone soy agar) medium with 0.2% glycerol. Cultures were incubated at 30 °C for several weeks to allow the development of slow growing species. Samples for nucleic acid analyses were preserved in the laboratory at −80 °C until being processed.

Only one strain producing violet pigments (MCs36) was obtained from the 2012 sampling. Previously, a few more sampling campaigns were carried out seeking for the isolation of this type of bacteria. Only in the 1997 (strain MC48) and 2005 (strain MC05) campaigns we were able to isolate two more strains producing the pigment.

A few *Streptomyces* from culture collections were used in this study: *Streptomyces parvus* DSM 40348^T, *Streptomyces pluricolorescens* DSM 40019^T, *Streptomyces cyaneofuscatus* DSM 41423^T, *Streptomyces vietnamensis* DSM 41927^T, *Streptomyces badius* DSM 40139^T, *Streptomyces sindenensis* DSM 40255^T, *Streptomyces globisporus* DSM 40199^T and *Streptomyces rubiginosohelvolus* DSM 40176^T. *Streptomyces coelicolor* A3(2) was kindly provided by Dr. Paloma Liras, Molecular Biology Department, University of Leon, Spain.

2.2. Field emission scanning electron microscopy

Small mortar fragments from the Circular Mausoleum tomb exhibiting green biofilms and violet stains were examined by field emission scanning electron microscopy (samples GB1, GV2 and VS3). Fresh samples were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate-buffer (pH 7.4) at 4 °C for 2 h and washed thrice in cacodylate-buffer. Subsequently, they were postfixed in 1% osmium tetroxide for 1 h at 4 °C and dehydrated by subsequent dilution series in ethanol and acetone finishing with 100% acetone before drying. The samples were dried in a critical point drying device (Leica EM 300) at 34.5 °C. Finally, the mortar samples were mounted on SEM stubs and sputter-coated with gold. FESEM examinations were performed in a FEI TENEO microscope, which was operated in secondary electron detection mode with an acceleration potential of 5 kV.

Fig. 1. Violet stains in the Circular Mausoleum, Roman Necropolis of Carmona, Spain. A. Entrance to the tomb. B. End of the tomb. C. Details of violet stains from A and neighbour phototrophic biofilms. D. Detail of violet stains from B. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)